International Journal of Medical Laboratory 2015;2(2):98-104.

International Journal of Medical Laboratory
Shahid Sadoughi University of Medical Sciences

Original Article

The Effect of Nonpolar Fraction of *Carum Copticum* Essence on Acetylcholine Induced Contraction in the Ileum of Isolated Rats

Seyed Hassan Hejazian[1]Ph.D., Sara Sadegh Zade[2]M.Sc., Mahila Lotfi[3] M.Sc., Fatemeh Safari[2*]Ph.D.

[1]Department of Physiology, Herbal Medicine Research Center, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[2]Department of Physiology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[3]Department of Physiology, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Article history
Received 15 Apr 2015
Accepted 8 Jun 2015
Available online 8 Aug 2015

Key words
Acetylcholine
Carum copticum
Ileum
Spasmolythic effect

A B S T R A C T

Background and Aims: Our previous study has demonstrated that essence of Carum copticum reduces contraction activity of ileum in rat. The present study was designed to find out the effects of nonpolar fraction of Carum copticum essence (NFCCE) on mechanical activity of the isolated ileum in rat.

Materials and Methods: For evaluation of spasmolythic property of fraction, different doses of the solution were added to organ bath after acetylcholine with concentration of 10-4 molar (M), and for assessment of antispasmodic effect of fraction, different doses of the solution were added to the organ bath before acetylcholine with concentration of 10-9 up to 10-2 M. Then isotonic contractions of ileum were recorded through an isolated tissue chamber in an organ bath using oscillographic device.

Results: Our findings showed that the spasmolytic effect of NFCCE in concentrations of 25, 50, and 100 ng/ml significantly reduces acetylcholine (10-4M)-induced contractions ($p< 0.05$). Also antispasmodic effect of NFCCE on logarithmic concentrations of acetylcholine (10-9 up to 10-2M) indicates that in the presence of 10-3 M acetylcholine, the maximum (100%) and minimum (84.9%), inhibition of contraction is induced by concentrations of 100 ng/ml and 25 ng/ml, respectively ($p< 0.05$).

Conclusions: The spasmolytic effect of this study is probably resulted by anti-cholinergic response of NFCCE on isolated ileum of rats.

[*] **Corresponding Author:** Department of Physiology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. **Email address**: fa.cardio@gmail.com

Introduction

Carum copticum L (Sprague ex turrill) is a plant of umbelliferae family with a white flower and small brownish seeds. Its major component is essence which is mainly composed of thymol, γ-terpinene, p-cymene, β-pinene, myrcene, and limonene [1]. It has been traditionally used in the treatment of many gastrointestinal disorders such as indigestion, colic, and diarrhea [2]. The extracts obtained from the seeds of C*arum copticum* have several pharmacological effects including anticholinergic [3], analgesic [4], anti-asthmatic [5] and antitussive [6] activity. The essential oil derived from extract reduces acetylcholine-induced contractions which is probably due to the thymol, the main constituent of the essential oil [7] and provokes the scientific investigators to evaluate the therapeutic effects of medicinal plants and their fractions. In our previous studies, the relaxant effect of C*arum copticum* water extract on intestinal motility [8], and its inhibitory effect on acetylcholine-induced contraction have been demonstrated on isolated ileum in rat [9]. Since the majority of pharmacologically active constituents of C*arum copticum* have been accumulated in its essential oil [1], this study was conducted to examine the spasmolytic and antispasmodic effect of nonpolar fraction (80% Hexan, 20% Chloroform) of *Carum copticum* essence (NFCCE) on acetylcholine-induced contraction in isolated ileum of rats.

Materials and Methods

In this study, 10 adult male albino rats weighing 200-250 g, living in the standard environmental and feeding conditions, were used for isolation of their ileum. For each experimental procedure totally 5 sample were picked up by the permission of the Animal Ethics Committee of Shahid Sadoughi University of Medical Sciences (Yazd, Iran), which was in accordance with the internationally accepted principles for laboratory animal use and care cited by the European Community guidelines. *Carum copticum* seeds were provided by Agricultural Research Center (Yazd, Iran) and identified by a botanist in this center. 100 gram of air-dried seeds of C*arum copticum* were gently grounded and mixed with 500 ml of double-distilled water. Then, its essence was prepared by Clevenger (Apparell de type Clevenger). The concentration of essence was 1.5% V/V. A sample of the yielded essence was analyzed by Agilent Technologies (Delaware, USA) 6890N network GC system (Delaware, USA). In this study, the 25, 50, and 100 ng/ml concentrations of NFCCE were examined for their spasmolytic and antispasmodic action. Acetylcholine chloride, as a standard stimulant of gastrointestinal smooth muscle was purchased from Sigma Aldrich Chemie Gmbh, Germany. Experiments were performed as described in our previous report [10]. Briefly, adult male albino rats were sacrificed by cervical dislocation. Segments of ileum (2 cm in length), were excised, flushed of their

contents, and trimmed of their mesentery. The specimens were conserved in Tyrode's solution until the onset of experimental procedure. The tissue sample was fixed at the bottom of the internal chamber of an organ bath (Organ bath 61300 biosciense UK) containing 50 ml Tyrode's solution in the axis of its longitudinal muscle and its opposite end was tightly tied to the isotonic transducer (the bioscience 400 Series Washington ocillograph) lever with a piece of thread, the chamber was maintained at 37 °C, and bubbled with 95% O2 and 5% CO2. Isotonic responses were recorded by using an isotonic transducer (T2) and an oscillograph recording system (the Bioscience 400 Series Washington Oscillograph). Then, it was allowed to stabilize for 15 minute prior to the addition of drug, and washed out in 30 minute-intervals by a fresh Tyrode's solution.

To investigate the spasmolytic action of NFCCE, first we obtained the maximum contraction of ileum using acetylcholine 10^{-4} M. Then, we studied the effect of different concentrations of essence on maximal contraction. Furthermore, to determine the antispasmolytic action of NFCCE, we applied different concentrations of essence to the tissues under study 7 minutes prior to applying the logarithmic concentrations of acetylcholine (10^{-9} up to 10^{-2}M) on the tissues to see the effect of the essence on the prevention of acetylcholine contraction. The Ethics Committee of Shahid Sadoughi University of Medical Sciences approved the study.

Statistical Analysis

The effect of different experimental solutions were expressed as Mean ± SD of percentage inhibition of contraction amplitude, and compared to maximum effect induced by acetylcholine. All statistical analyses and comparisons were made by means of the ANOVA followed by Tukey's test. The statistical significance was considered as $P< 0.05$.

Results

The chemical constituents of the essence from distillate extract of C*arum copticum* seeds were studied by gas chromatography mass spectrometry (GC-MS). The results showed that there were numerous ingredients in the sample, and thymol was its main constituent (Table 1). The inhibitory effect of NFCCE (100ng/ml) on acetylcholine-induced contraction was immediately initiated upon its addition to the organ bath, when it reached to its maximum within 0.5 minute and persisted at least for five minutes.

Table 1. Chemical constituents of nonpolar fraction (80% Hexan, 20% Chloroform) of *Carum Copticum* essence

NO.	Compound	Composition (%)	Retention index
1	o-Cymene	0.139	939
2	Eucalyptol	0.126	945
3	gamma-Terpinene	0.058	975
4	4-Terpineol	2.152	1078
5	alpha-Terpineol	0.278	1101
6	Thymol	72.740	1207
7	Carvacrol	3.556	1210
8	Dimetyl phentalate	2.411	1331
9	Dibotyl phentalate	3.402	1770

Our findings showed that the spasmolytic effect of NFCCE in concentrations of 25, 50, and 100 ng/ml reduced acetylcholine (10-4M)-induced contractions significantly by 92± 0.34, 100± 0.00, and 100±0.00, respectively vs. 100 ±0.00 ($p< 0.05$, Fig. 1).

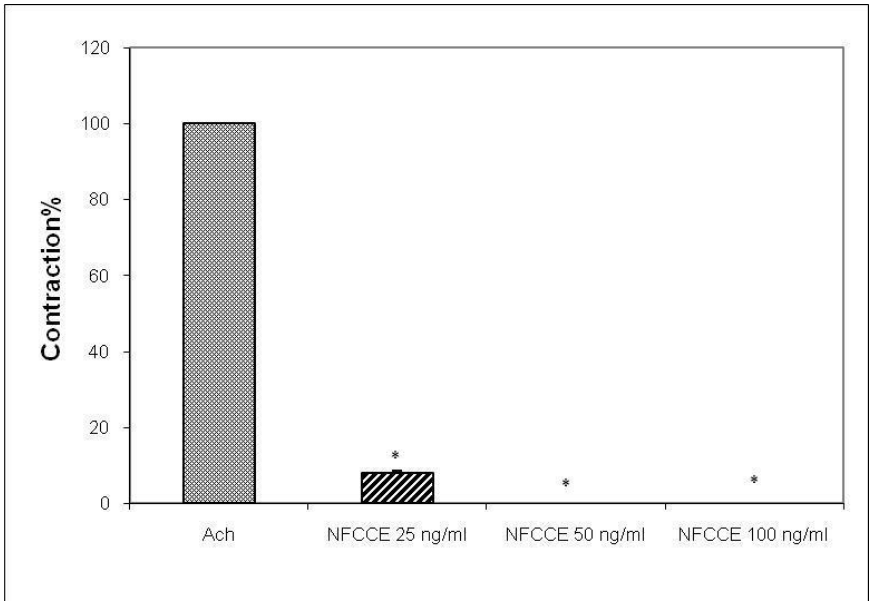

Fig. 1. Spasmolytic effect of NFCCE on acetylcholine-induced contractions (10-4 M) in the isolated ileum in rat (N=6). *indicates the significant difference ($p< 0.05$) as compared to the acetylcholine-induced contraction according to the one-way ANOVA fallowed by Tukey's post-test.

The antispasmodic effect of NFCCE on isolated ileum of the rat indicates the significant difference between the acetylcholine-induced contractions in the presence of saline and three different concentrations of essence ($p<0.05$). In the presence of 10^{-3}M acetylcholine, the maximum (100%) and minimum (84.9%) inhibition of contraction were induced by concentrations of 100 and 25 ng/ml, respectively, and the difference between the antispasmodic effects of these two concentrations was statistically significant ($p< 0.05$, Fig. 2).

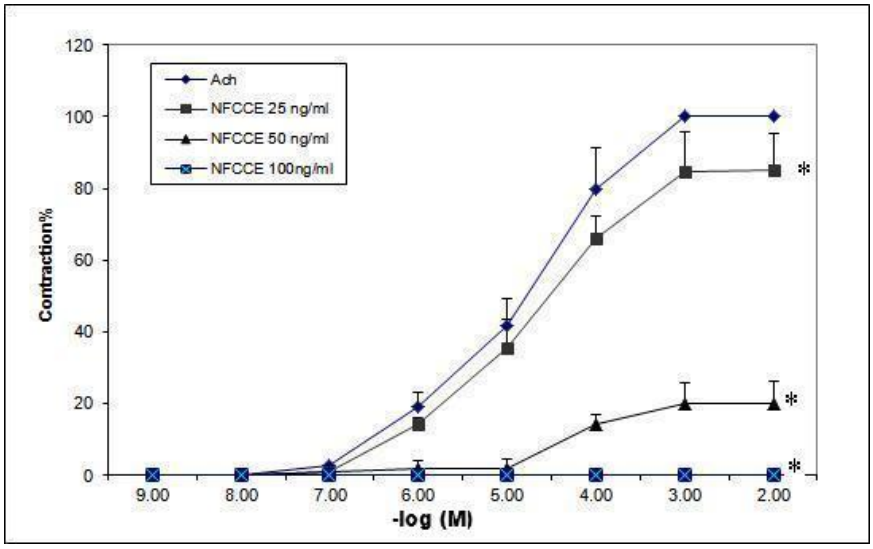

Fig.2. Antispasmodic effect of NFCCE onileum of isolated rats. Each point indicates the mean of 5 experiments and the vertical bars represent the SD. *indicates the significant difference between the acetylcholine-induced contractions in the presence of saline and 3 different concentrations of essence ($p< 0.05$) using one-way ANOVA followed by Turkey's post-test.

Discussion

Our findings indicated that different concentrations of NFCCE can inhibit exert a potent spasmolytic and antispasmodic effect on isolated ileum of the rats. The result was consistent with our previous study in which the C*arum copticum* essence showed a significant relaxant effect on ileal smooth muscle [7]. However, the essence had more potent inhibitory effect on acetylcholine-induced contractions as compared to NFCCE. In our previous study, the time course for essence to exert its spasmolytic action was shorter than that of NFCCE. The essence exerted its inhibitory effect immediately and reached its maximum rate within 30 seconds while the NFCCE initiated its inhibitory action on acetylcholine-induced contraction after one minute and completed its action within two minutes (9). The NFCCE in our sample mainly consisted of thymol, cymene, gamma-terpinene, carvacrol, and pinenes. These compounds have also been previously reported as the major constituents of C*arum copticum* extracts [10]. The relaxation of smooth muscle was induced by different mechanisms that include the blocking action on excitatory/inhibitory pathways such as cholinergic [11], histaminergic [12], adrenergic [13], purinergic [14], GABAergic [15], and/or nitric oxide [16]. On the basis of our previous study in which the essence showed a significant muscle relaxantion effect [7] and also regarding the present study in which the NFCCE had more potent antispasmodic effect, it should be concluded that the observed inhibitory effect of essence on smooth muscle contraction may be through the thymol action. There is also reported that C*arum copticum* extract affects the tracheal smooth muscle via

their anti-cholinergic [17], anti histaminic [18], or calcium channel blocking activity [19]. These reports have indicated that the anti-cholinergic property of its essence and NFCCE could be indirectly related to β-adrenergic stimulatory action or may be due to their direct inhibitory effect on cholinergic receptors [17]. This is correct that contraction of smooth muscle is depend on calcium availability, and thymol exerts its relaxant effect through opposing this process [20]. The inhibitory effect of NFCCE of this plant on isolated ileum preparations in our study indicates its functional antagonistic effect on cholinergic receptors in rats ileum. It can also block the Ca^{+2} influxes through the cell membrane [21] and reduction of the calcium content of the sarcoplasmic reticulum [22]. The carvacrol is also another pharmacological constituent in the essence and has shown a significant relaxant effect on smooth muscles by blocking muscarenic receptors and/or β-adrenergic stimulation [23]. Since these inhibitory compounds are the major constituent of our samples of *NFCCE*, the relaxant effect observed in this study is probably due to this agent which can exert an anti-cholinergic property.

Conclusion

Our results showed that the spasmolytic effect of NFCCE in different concentrations reduces acetylcholine-induced contractions. The spasmolytic effect is probably resulted by anti-cholinergic response of NFCCE on isolated ileum of rats.

Conflict of Interest

None declared

Acknowledgement

The authors give thanks to the Research Deputy of Shahid Sadoughi University of Medical Sciences Yazd, Iran as the sponsor of this research.

References

[1]. Khajeh M, Yamini Y, Sefidkon F, Bahramifar N. Comparison of essential oil of Carum Copticum obtained by supercritical carbon dioxide extraction and hydrodistillation methods. Food Chem. 2004; 86: 587–91.

[2]. Avesina, Law in Medicine vol. 2, Soroush Press, Tehran, p. 187, 985.

[3]. Devasankaraiah G, Hanin I, Haranath Ps, Ramanamurthy PS. Cholinomimetic effects of aqueous extracts from *Carum Copticum* seeds. Br J Pharmacol. 1974; 52(4): 613–14.

[4]. Dashti-Rahmatabadi MH, Hejazian SH, Morshedi A, Rafati A. The analgesic effect

of *Carum Copticum* extract and morphine on phasic pain in mice. J Ethnopharmacol. 2007; 109: 226-28.

[5]. Boskabady MH, Alizadeh M, Jahanbin B. Bronchodilatory effect of Carum Copticum in airways of asthmatic patients. Therapie. 2007; 62(1): 23-29.

[6]. Boskabady MH, Jandaghi P, Kiani S, Hasanzadeh L. Antitussive effect of Carum Copticum in guinea pigs. J Ethnopharmacol. 2005; 97:79-82.

[7]. Hejazian SH, Sepehri H, Dashti MH, Mahdavi SM. Does essential oil of Carum copticum affect acetylcholine-induced contraction in isolated rat's Ileum? African Journal of Pharmacy and Pharmacology. 2011; 5: 1432-435.

[8]. Hejazian SH, Morowatisharifabad M, Mahdavi SM. Relaxant effect of Carum Copticum on intestinal motility in ileum of rat, World Journal of Zoology 2007; 2:15-18.

[9]. Hejazian SH, Dashti-Rahmatabadi MH, Mahdavi SM. The effect of Carum Copticum extract on acetylcholine induced contraction in isolated rat's ileum. J Acupuncture Meridian Stud. 2009; 2:275-78.

[10]. Hejazian SH, Bagheri SM, Safari F. Spasmolytic and Antispasmodic action of Trachyspermum ammi Essence on Rat's Ileum Contraction. North american medical sciense 2014; 6:643-47.

[11]. Unno T, Matsuyama H, Izumi Y, Yamada M, Wess J, Komori S. Roles of M2 and M3 muscarinic receptors in cholinergic nerve-induced contractions in mouse ileum studied with receptor knockout mice. Br J Pharmacol. 2006; 149: 1022-30.

[12]. Sá-Nunes A, Corrado AP, Baruffi MD, Faccioli LH, Disodium cromoglycate prevents ileum hyperreactivity to histamine in Toxocara canis-infected guinea pigs. Pharmacol Res. 2003; 48(5): 451-55.

[13]. Roberts SJ, Papaioannou M, Evans BA ,Summers RJ. Characterization of beta-adrenoceptor mediated smooth muscle relaxation and the detection of mRNA for beta1-, beta-2- and beta3-adrenoceptors in rat ileum. Brit. J. Pharmacol. 1999; 127(4): 949–61.

[14]. Van Crombruggen K, Van Nassauw L, Timmermans JP, Lefebvre RA. Inhibitory purinergic P2 receptor characterisation in rat distal colon. Neuropharmacology. 2007; 53:257-71.

[15]. Zizzo MG, Mulè F, Serio R. Functional evidence for GABA as modulator of the contractility of the longitudinal muscle in mouse duodenum: role of GABA (A) and GABA(C) receptors. Neuropharmacology 2007; 52:1685-90.

[16]. Kito Y, Suzuki H. Effects of Dai-kenchu-To on spontaneous activity in the mouse small intestine. J Smooth Muscle Res. 2006; 42: 189-201.

[17]. Boskabady MH, Moetamedshariati V. Bronchodilatory and anti-cholinergic effects of Carum Copticum on isolated guinea-pig tracheal chain. Eur Respir J. 1996; 23, 28s.

[18]. Boskabady MH, Shaikhi J. Inhibitory effect of Carum Copticum on histamine (H1) receptors of isolated guinea-pig tracheal chain. J. Ethnopharmacol. 2000; 69(3): 217-27.

[19]. Gilani A.H, Jabeen Q, Ghayur MN, Janbaz KH, Akhtar MS. Studies on the antihypertensive,antispasmodic,bronchodilator and hepatoprotective activities of the Carum Copticum seed extract. J. Ethnopharmacol. 2005; 98:127-135.

[20]. Hejazian SH, Bagheri SM, Fattahi A. Role of thymol in inhibition of acetylcholine induced contraction of isolated Rat's Ileum. Physiology and pharmacology 2013;17: 216-23.

[21]. Ceccatto VM, Coelho-de-Souza AN, Gomes MD, Lahlou S, Leal-Cardoso JH, Lima FC, et al. Vasorelaxant effect of the monoterpenic phenol isomers, carvacrol and thymol, on rat isolated aorta. Fundam Clin Pharmacol. 2009; 24: 341-50.

[22]. Szentandrássy N, Szigeti G, Szegedi C, Sárközi S, Magyar J, Bányász T, et al. Effect of thymol on calcium handling in mammalian ventricular Myocardium. Life Sci. 2004; 74(7): 909-21.

[23]. Boskabady MH, Ramazni M, Tabai T. Relaxant effects of different fractions of essential oil from Carum Copticum on gunea pig tracheal chains. Phytother Res. 2003; 17(10): 1145-149.